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Report of Three Novel Germline *CYLD* Mutations in Unrelated Patients with Brooke-Spiegler Syndrome, Including Classic Phenotype, Multiple Familial Trichoepitheliomas and Malignant Transformation

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Key Words
Adnexal tumors · Spiradenoma · Spiradenocylindroma · Cylindroma · Trichoblastoma · Trichoepithelioma · *CYLD* gene · Carcinoma

Abstract
Brooke-Spiegler syndrome is a rare autosomal-dominant genetic disorder characterized by multiple adnexal tumors, including cylindromas, spiradenomas, spiradenocylindromas and trichoepitheliomas. It is caused by germline *CYLD* mutations commonly leading to a premature stop codon. We here report on 3 novel *CYLD* mutations in 3 unrelated BSS patients, including the classic phenotype, multiple familial trichoepitheliomas phenotype and malignant transformation. These included c.1821_1826+1delinsCT/L607Ffs*9, c.2666A>T/p.D889V and c.2712delT/p.905Kfs*8. By extending the spectrum of *CYLD* mutations, better understanding of the molecular mechanisms of BSS can be gained, which might later assist in finding new treatment options.

Introduction

Brooke-Spiegler syndrome (BSS) is a rare autosomal-dominant genetic disorder characterized by multiple adnexal tumors, including cylindromas, spiradenomas, spiradenocylindromas and trichoepitheliomas (cribriform trichoblastomas). The tumors are mostly localized in the head and neck area and may undergo a malignant transformation in 5–10% of cases [1–4]. In addition, BSS patients may very rarely develop tumors of the major (parotid) and minor salivary glands, and exceptionally in the breast [5–7].

Familial cylindromatosis and multiple familial trichoepitheliomas (MFT) are nowadays considered phenotypic variants of BSS: 1 patient may develop various types of tumors and/or different phenotypes may occur in the same family [8–16]. All three conditions are caused by germline mutations in the same *CYLD* gene [17–20].

At the time of writing, a total of 95 distinct germline *CYLD* mutations have been reported [17, 21–28]. *CYLD* is a tumor suppressor gene located on chromosome 16q

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Fig. 1. Patient 1. Multiple skin-colored papules on the face favoring the nasolabial folds and eyelids (covered).

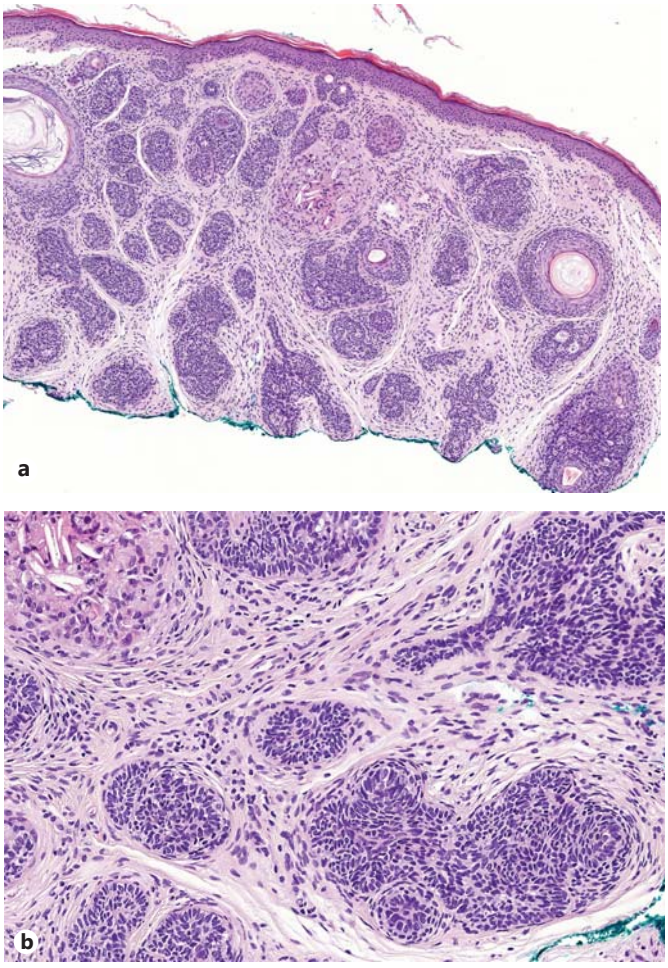

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Fig. 2. a, **b** Patient 1. Cribriform trichoblastoma (trichoepithelioma). Cribriform and solid basaloid cell aggregates focally resembling follicular germ structures surrounded by specific follicular stroma resembling follicular papillae.

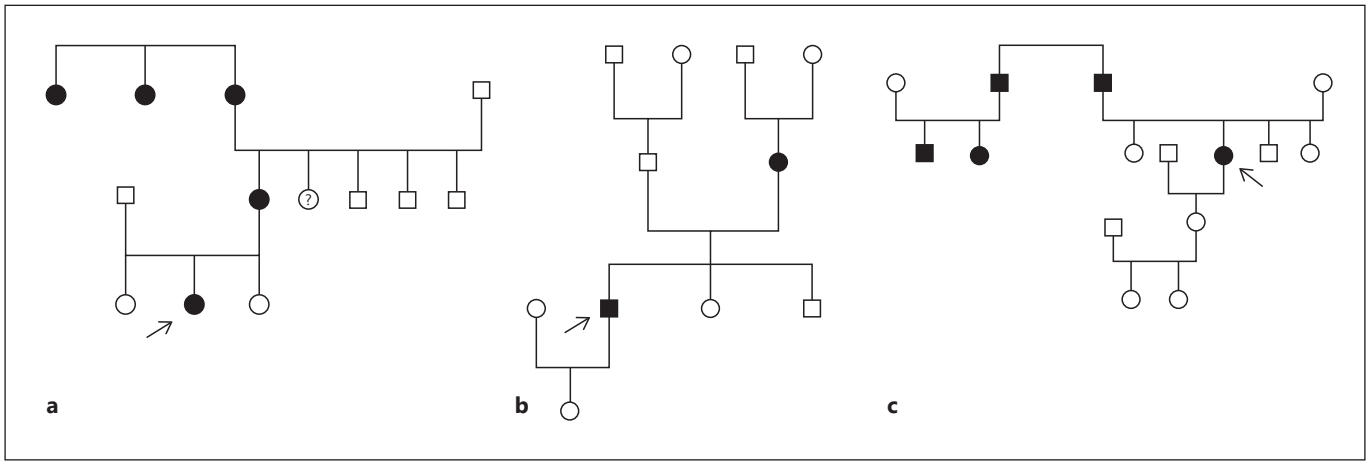

Fig. 3. Family pedigree of patient 1 (**a**), patient 2 (**b**) and patient 3 (**c**).

Table 1. Main clinicopathological data

Case	Sex/age, years	Nationality/ethnicity	Clinical features	Available histology	*CYLD* mutation
1	F/29	Swiss	multiple paranasal papules	2 trichoepitheliomas	c.2666A>T
2	M/60	Czech	multiple confluent scalp tumors, the biggest one measuring 5 × 3 × 2.5 cm, with recent rapid growth	1 BCAC-HG evolving from spiradenocylindroma; 1 spiradenoma; 1 cylindroma	c.2712delT
3	F/76	Slovak	multiple tumors on the face and scalp	2 spiradenocylindromas; 1 trichoepithelioma	c.1821_1826+1delinsCT

which has 20 exons and encodes a protein that negatively regulates the NF-κB and JNK pathways [29]. Most germline mutations reported to date are frameshift and nonsense mutations commonly leading to a premature stop codon. We here report on 3 BSS patients (including 1 with a malignant phenotype) from 3 unrelated families with novel germline *CYLD* mutations, thus extending the catalogue of known germline *CYLD* mutations.

Patients and Methods

Patients

The main clinical data, histopathological and molecular findings are summarized in table 1.

Patient 1. A 29-year-old woman presented with a few papules on her face, favoring the nasolabial folds and eyelids. She started developing them at the age of 13 years (fig. 1). A shave biopsy of two papules revealed cribriform trichoblastoma (trichoepithelioma) in each of the specimens (fig. 2). The family pedigree showed an autosomal dominant mode of inheritance (fig. 3a).

Patient 2. A 60-year-old man had multiple partly confluent tumors on his scalp (fig. 4) which first appeared at the age of 40 years. Some of the tumors had grown faster in the last 2 years, with the largest one (right temporal area) reaching the size of 5 × 3 × 2.5 cm. Two out of four specimens showed stereotypical features of a cylindroma and a spiradenoma. The other two specimens revealed malignant transformation of a preexisting spiradenocylindroma featuring nests and sheets of medium-sized pleomorphic basaloid cells with an infiltrative growth pattern and plentiful mitoses. The stroma was partly fibrous, partly myxoid with a retraction phenomenon. Lymphocytes were missing and the malignant moiety of the tumor was diffusely positive for p53 (fig. 5, 6). The mother of the patient suffered from similar tumors on her scalp (fig. 6b).

Patient 3. A 76-year-old woman presented with multiple skin tumors on her face and scalp, which had appeared at the age of 17 years. Two biopsies were taken, which revealed a spiradenocylindroma and cribriform trichoblastoma (trichoepithelioma) next to an incipient spiradenocylindroma. Family members were affected in an autosomal-dominant fashion (fig. 3c).

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Fig. 4. Patient 2. Multiple variably sized red to bluish confluent nodules on the scalp.

Molecular Analysis

After obtaining the patients' informed consent, analysis of germline *CYLD* mutations was performed as described previously [30]. Briefly, DNA was extracted from peripheral blood using a QIAsymphony DNA Mini Kit (Qiagen, Hilden, Germany) on an automated extraction system (QIAsymphonySP, Qiagen) according to the manufacturer's protocol. Coding sequences and

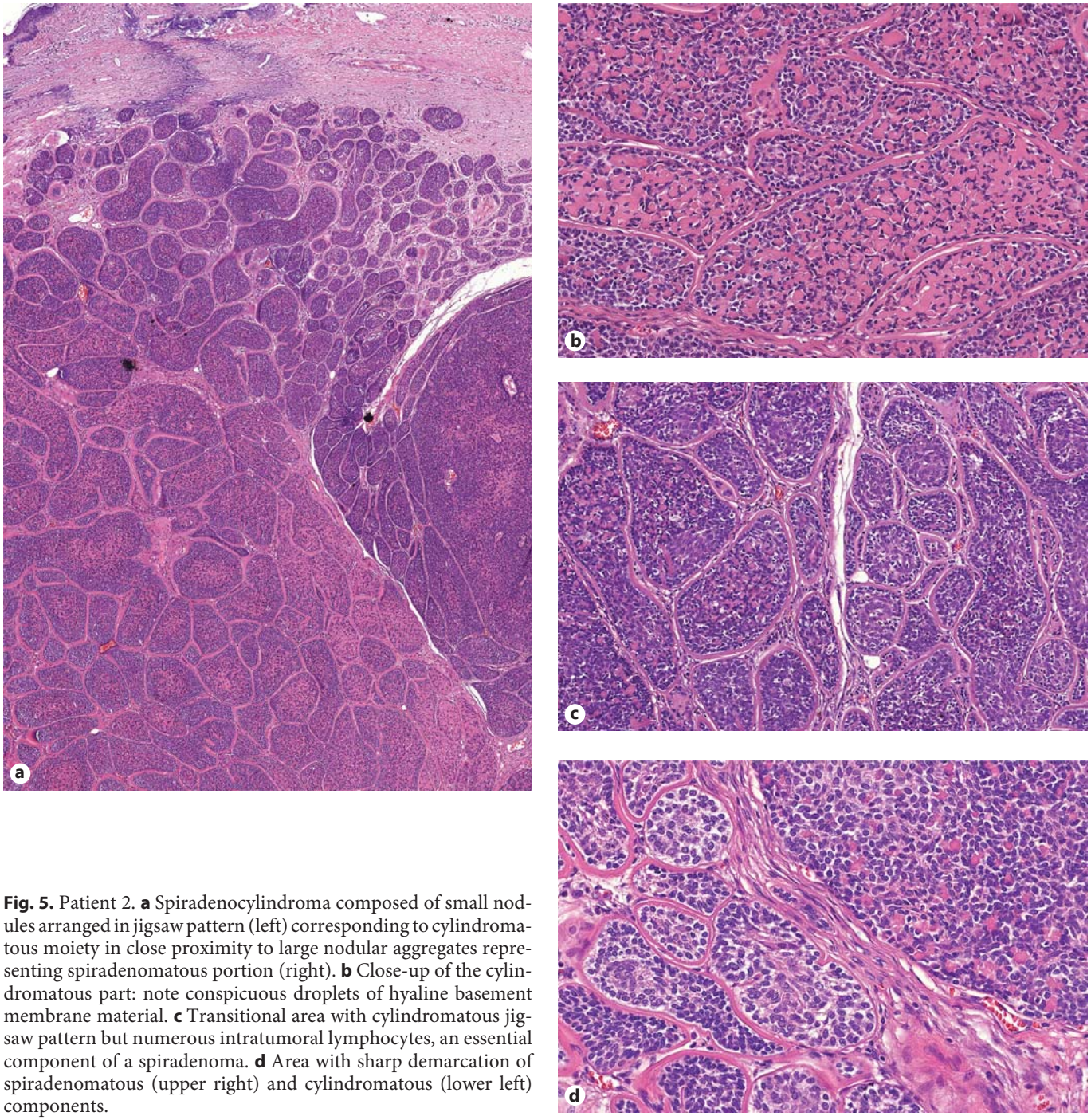

Fig. 5. Patient 2. **a** Spiradenocylindroma composed of small nodules arranged in jigsaw pattern (left) corresponding to cylindromatous moiety in close proximity to large nodular aggregates representing spiradenomatous portion (right). **b** Close-up of the cylindromatous part: note conspicuous droplets of hyaline basement membrane material. **c** Transitional area with cylindromatous jigsaw pattern but numerous intratumoral lymphocytes, an essential component of a spiradenoma. **d** Area with sharp demarcation of spiradenomatous (upper right) and cylindromatous (lower left) components.

exon-intron junctions were amplified by HotStar Taq DNA Polymerase (QIAntigen, Hilden, Germany). PCR products were purified and sequenced bidirectionally, using the Big Dye Terminator Sequencing Kit (Applied Biosystems, Carlsbad, Calif., USA). In silico analysis of the impact of the mutation on protein structure and function was performed by PolyPhen-2 and PROVEAN.

Results

Three novel *CYLD* gene mutations were found and the proteins were in silico predicted. These included c.1821_1826+1delinsCT/L607Ffs*9, c.2666A>T/p.D889V and c.2712delT/p.905Kfs*8 (fig. 7). The prediction of

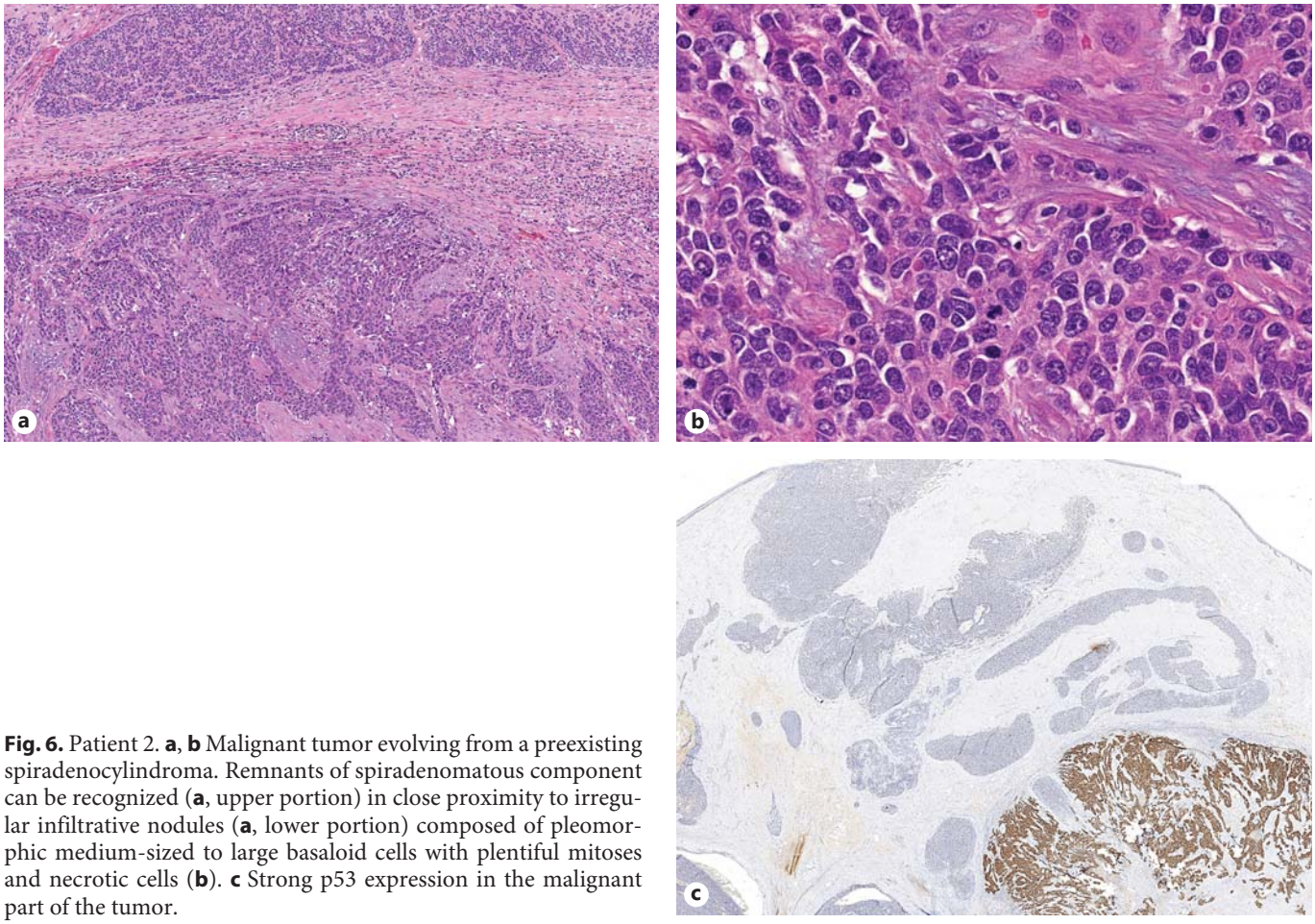

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Fig. 6. Patient 2. **a**, **b** Malignant tumor evolving from a preexisting spiradenocylindroma. Remnants of spiradenomatous component can be recognized (**a**, upper portion) in close proximity to irregular infiltrative nodules (**a**, lower portion) composed of pleomorphic medium-sized to large basaloid cells with plentiful mitoses and necrotic cells (**b**). **c** Strong p53 expression in the malignant part of the tumor.

protein functionality by PolyPhen-2 and by PROVEAN revealed a malignant status of missense mutation ('probably damaging', score 0.999, and 'deleterious', respectively).

Discussion

In this study, we have reported three novel *CYLD* mutations in 3 unrelated BSS patients. Our patients have a variable phenotypic expression ranging from tiny papules on the face representing trichoepitheliomas in patient 1 (MFT phenotype) to multiple tumors on the scalp in patients 2 and 3. Additionally, in patient 2, malignant transformation of spiradenocylindroma has occurred, and the tumor was classified as a salivary gland-type basal cell adenocarcinoma-like pattern, high-grade (BCAC-HG). In a series of 24 malignant tumors evolving from preexisting benign spiradenoma, cylindroma and spiradenocylindroma, both sporadic and associated with BSS, this was the second most common type of a malignant pattern after low-grade salivary gland-type BCAC [31]. In the same study, a correlation between the histologic pattern and clinical course was found in the sense that in patients with BCAC-HG, a more aggressive course is to be expected. The malignant portion was strikingly positive for p53, as is sometimes seen in these lesions [32].

CYLD has no mutation hotspot, but the three novel germline mutations we identified are located in the usually mutated parts of the *CYLD* gene, namely between exons 9 and 20 (fig. 8) [27]. Two mutations represent a splice-site (patient 1) and a frameshift (patient 3) mutation, resulting in a premature stop codon and, thus, substantially disturbing the protein structure. In patient 1, a missense mutation was found, meaning that a change in a single amino acid had occurred. Mutations that change an amino acid can differ in their impact on protein func-

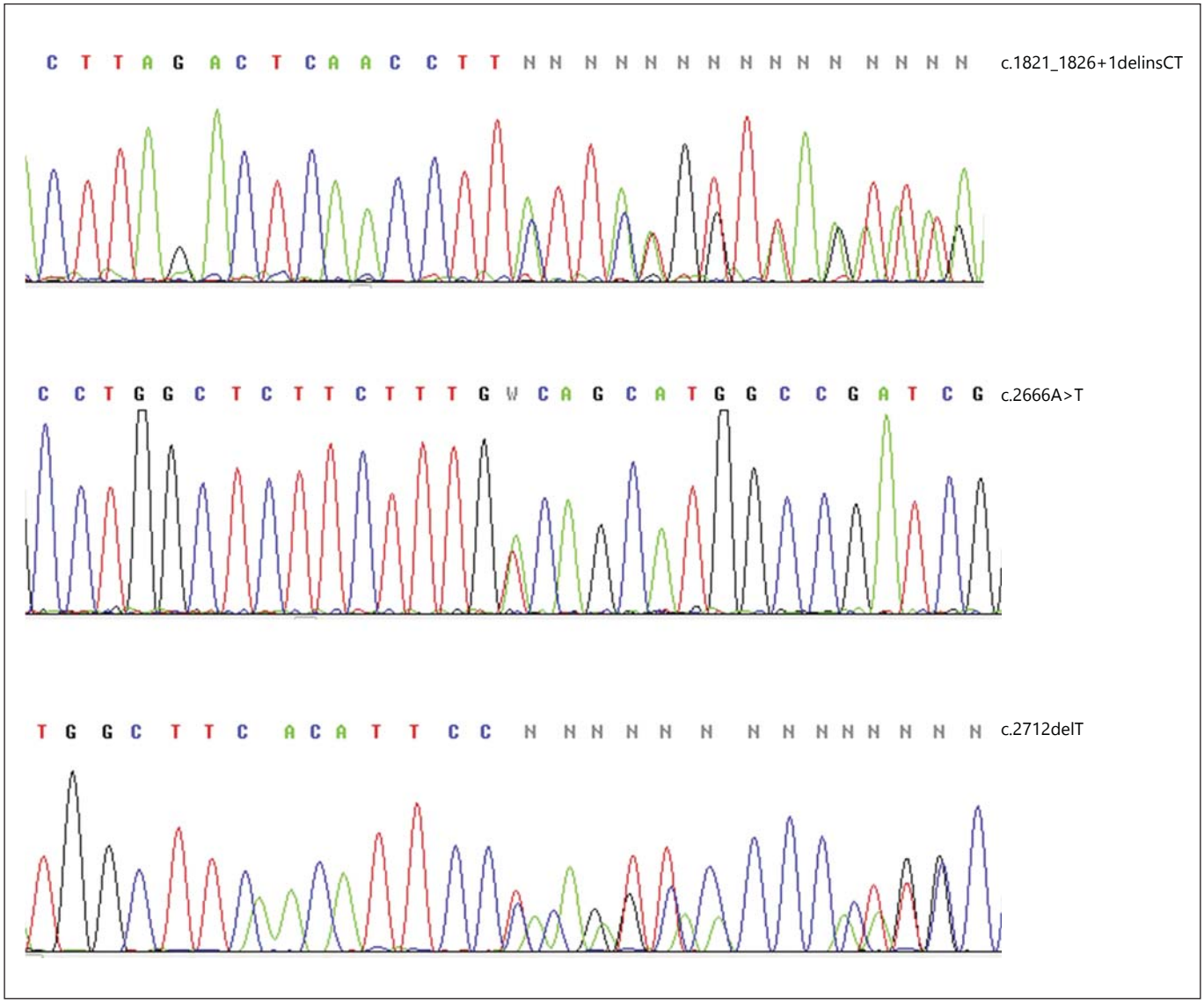

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Fig. 7. Sequences of the three novel mutations identified in our study (c.1821_1826+1delinsCT/L607Ffs*9 (case 3), c.2666A>T/p.D889V (case 1) and c.2712delT/p.905Kfs*8 (case 2).

tion. In our case, the in silico analysis supports the claim of causality of this mutation.

Using the current analysis (sequencing of coding sequences and exon-intron junctions), germline *CYLD* mutations are presently detected in about 76% of cases (the mutation frequency is about 85% for classic BSS and 44% for MFT) [17, 33]. Mutations in introns leading to intronic exonization and large deletions have been reported in some of those 'negative' cases [30, 34]. So far, no genotype-phenotype correlation has been found [11, 17, 35]. Bigger cohorts with accurate correlation of molecular, clinical and histomorphologic data may eventually help in developing prognostic criteria. By extending the spectrum of *CYLD* mutations a better understanding of the molecular mechanisms of BSS can be gained. This might later assist in identifying new treatment options.

Disclosure Statement

The authors have nothing to disclose to the editor regarding any direct or indirect financial implication that publication of this paper may have for them, their relatives or their institution.

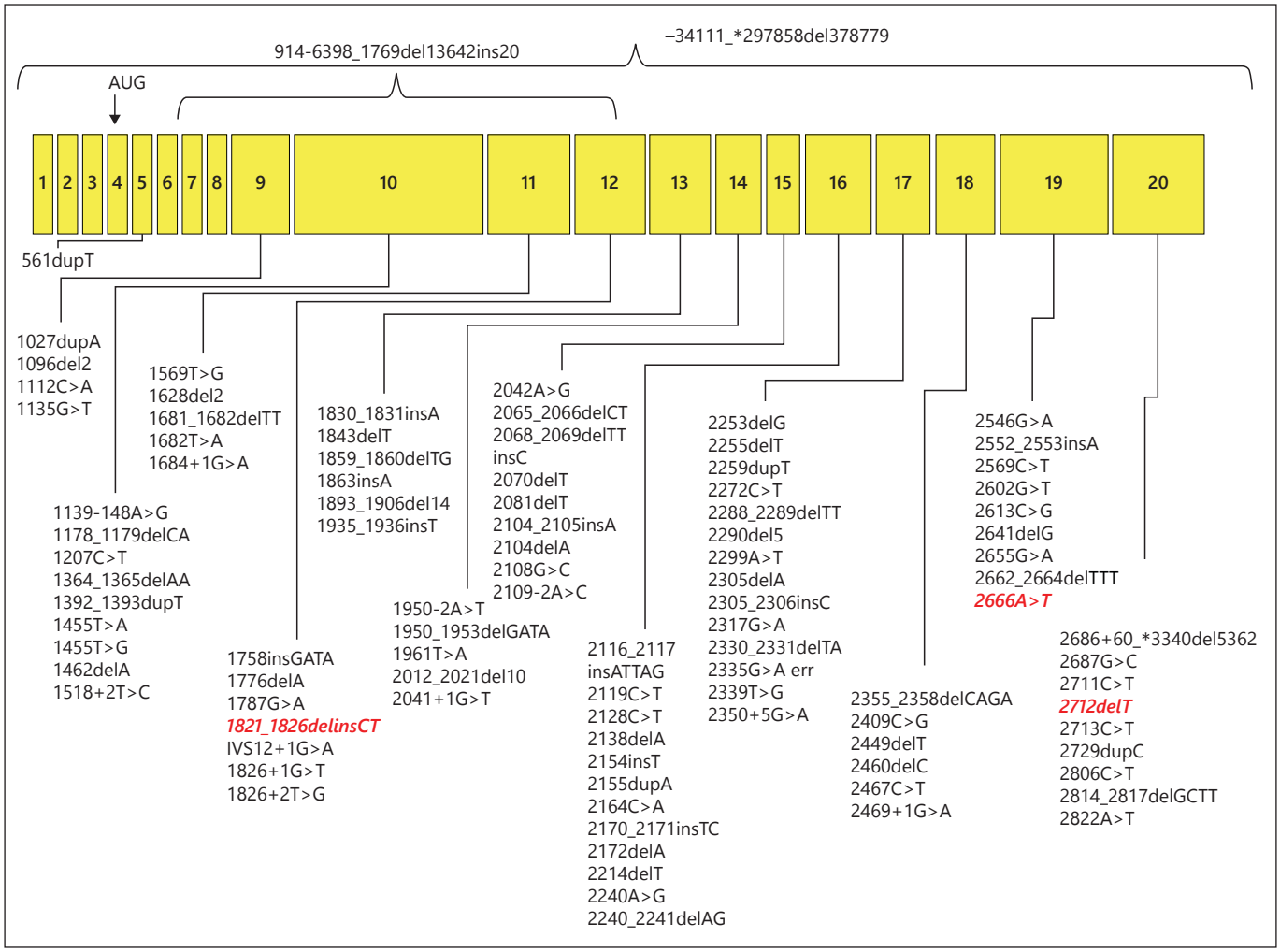

Fig. 8. Presently identified germline mutations and their distribution in the *CYLD* gene. Novel mutations are identified in red (modified from Grossmann et al. [17]).

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